FAMILY: HYDROPHYLLACEAE
Genera: Hydrophyllum, Phacelia

1. PRECONDITIONING:

<table>
<thead>
<tr>
<th>METHOD</th>
<th>TIME (h)</th>
<th>TEMP (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imbibe on moist media</td>
<td>overnight</td>
<td>20-25</td>
</tr>
</tbody>
</table>

Morphology

![Phacelia](image1) ![Hydrophyllum](image2)

<table>
<thead>
<tr>
<th><img src="image3" alt="Phacelia" /> <img src="image4" alt="Hydrophyllum" /></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig 1 External</td>
</tr>
<tr>
<td>Fig 2 Embryo</td>
</tr>
</tbody>
</table>

2. PREPARATION AND STAINING:

<table>
<thead>
<tr>
<th>METHOD</th>
<th>TZ Conc (%)</th>
<th>TIME (h)</th>
<th>TEMP (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bisect longitudinally and retain half for staining or leave enough intact to keep both halves together</td>
<td>1</td>
<td>24-48</td>
<td>30-35</td>
</tr>
</tbody>
</table>

Note: Embryo tends to separate from seeds that have been completely bisected.

![deep cut with some intact tissue to keep both halves together](image5)

![longitudinal cut](image6)

Fig 3 Preparation method
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Post-staining notes: None or spread halves apart to evaluate embryo.

3. EVALUATION:

VIABLE (NORMAL STAINING)
- embryo staining red or orange acceptable
- yellow embryos often only stained on cut surface
- endosperm is living and must stain

NON-VIABLE (ABNORMAL OR NO STAINING)
- embryo staining pale orange to yellow not acceptable
- endosperm unstained

Notes: If embryo is pale orange or yellow, allowing additional staining time may bring about further color change.

Fig 4 Seed stain evaluation

Photos: Annette Miller, USDA/ARS NGRP